

Altered Glycosylation of Human Alpha-1-acid Glycoprotein as a Biomarker for Malignant Melanoma

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Supplementary Materials

Table S1. The glycan isomers identified in the melanoma and control samples. Retention time, mass accuracy, sialic acid linkage type and fucose positions (where available) are also given. In the glycan compositions N refers to N-acetylglucosamine, H to hexoses (galactose and mannose), S to sialic acid and F to fucose units. The digits after the letters refer to the number of each residue build up the molecule.

Glycan	Isomer	RT (min)	Mass error (ppm)	Sialic acid linkage	Fucose position
Biantennary					
N4H5SF	Isomer 1	43.49	2.57	α -2,3	core
	Isomer 2	44.71	-3.04	-	-
	Isomer 3	45.33	3.90	α -2,6	core
	Isomer 4	46.43	4.32	-	-
	Isomer 5	47.11	3.56	α -2,6	antenna
N4H5S2	Isomer 1	70.53	2.86	α -2,3	-
	Isomer 2	73.02	3.00	α -2,3, α -2,6	-
	Isomer 3	75.31	2.92	α -2,6	-
N4H5S2F	Isomer 1	67.93	7.40	α -2,3	core
	Isomer 2	69.26	13.22	-	-
	Isomer 3	69.99	8.32	α -2,3, α -2,6	core
	Isomer 4	70.60	12.73	-	-
	Isomer 5	71.16	9.39	-	-
	Isomer 6	72.28	8.57	α -2,3, α -2,6	antenna
	Isomer 7	73.50	14.75	-	-
Triantennary					
N5H6S	Isomer 1	43.94	4.07	-	-
	Isomer 2	44.90	4.16	-	-
	Isomer 3	45.27	3.86	α -2,6	-
	Isomer 4	46.06	5.95	-	-
	Isomer 5	46.67	4.17	α -2,6	-
	Isomer 6	47.11	1.79	α -2,6	-
N5H6S2	Isomer 1	65.94	1.56	-	-
	Isomer 2	66.80	2.57	-	-
	Isomer 3	67.41	2.15	-	-
	Isomer 4	68.57	0.48	α -2,6	-
	Isomer 5	69.39	1.41	α -2,6	-
	Isomer 6	70.06	2.46	α -2,6	-
	Isomer 7	71.13	2.51	-	-
	Isomer 8	71.72	1.94	-	-
N5H6S2F	Isomer 1	63.78	-4.98	-	-
	Isomer 2	66.15	-5.16	-	-
	Isomer 3	65.98	-4.87	-	-
	Isomer 4	67.62	0.02	α -2,6	antenna
	Isomer 5	69.32	0.48	α -2,6	antenna
	Isomer 6	70.92	1.01	-	-
N5H6S3	Isomer 1	106.35	2.74	-	-
	Isomer 2	108.49	4.05	α -2,6	-
	Isomer 3	111.10	1.73	α -2,6	-
N5H6S3F	Isomer 1	101.56	0.16	α -2,3, α -2,6	antenna
	Isomer 2	104.04	0.76	α -2,3, α -2,6	antenna
	Isomer 3	106.62	-3.14	-	-

N5H6S3F2	Isomer 1	96.54	6.62	-	-
	Isomer 2	99.08	3.39	-	-
	Isomer 3	102.40	3.61	-	-
	Isomer 4	106.30	-3.49	-	-
	Isomer 5	108.70	-4.46	-	-
	Isomer 6	112.60	0.43	-	-
N5H6S3F3	Isomer 1	94.10	-9.58	-	-
	Isomer 2	102.00	-8.98	-	-
	Isomer 3	104.23	-7.08	-	-
	Isomer 4	108.77	-6.81	-	-
Tetraantennary					
N6H7S	Isomer 1	44.44	0.97	-	-
	Isomer 2	45.06	-0.35	-	-
	Isomer 3	45.43	1.75	-	-
	Isomer 4	45.86	1.71	-	-
	Isomer 5	46.52	1.73	-	-
N6H7SF	Isomer 1	43.62	2.11	-	-
	Isomer 2	44.17	-1.30	-	-
	Isomer 3	44.56	8.65	-	-
	Isomer 4	44.94	4.29	-	-
	Isomer 5	45.66	2.93	-	-
	Isomer 6	46.38	2.44	-	-
	Isomer 7	47.04	6.94	-	-
N6H7S2	Isomer 1	63.28	4.27	-	-
	Isomer 2	65.07	4.33	-	-
	Isomer 3	65.66	3.32	α -2,3, α -2,6	-
	Isomer 4	66.83	3.94	α -2,3, α -2,6	-
	Isomer 5	67.54	3.97	α -2,6	-
N6H7S2F	Isomer 1	64.33	3.39	α -2,6	antenna
	Isomer 2	65.01	3.23	α -2,6	antenna
	Isomer 3	66.00	2.83	α -2,6	antenna
	Isomer 4	66.76	3.30	α -2,6	antenna
	Isomer 5	67.32	5.37	-	-
N6H7S3	Isomer 1	92.20	-0.91	-	-
	Isomer 2	94.31	1.65	-	-
	Isomer 3	95.87	1.07	-	-
	Isomer 4	96.76	-3.32	α -2,3, α -2,6	-
	Isomer 5	97.99	-3.50	α -2,3, α -2,6	-
	Isomer 6	99.27	-3.88	α -2,3, α -2,6	-
	Isomer 7	101.17	-2.98	α -2,6	-
N6H7S3F	Isomer 1	87.74	5.42	-	-
	Isomer 2	90.29	5.98	-	-
	Isomer 3	93.38	6.09	α -2,6	antenna
	Isomer 4	94.88	3.66	α -2,6	antenna
	Isomer 5	96.18	1.19	α -2,3, α -2,6	antenna
	Isomer 6	97.68	7.11	α -2,6	antenna
	Isomer 7	99.85	6.22	-	-
N6H7S4	Isomer 1	87.44	2.87	α -2,6	-
	Isomer 2	89.74	-3.40	-	-

	Isomer 3	103.90	-2.97	-	-
N6H7S4F	Isomer 1	83.46	1.81	-	-
	Isomer 2	93.77	3.25	-	-
N6H7S4F2	Isomer 1	99.39	3.67	-	-
	Isomer 2	108.76	5.36	-	-
N6H7S4F3	Isomer 1	90.15	-3.56	-	-
N7H8S3	Isomer 1	85.61	-1.5	-	-
	Isomer 2	88.24	4.83	-	-
	Isomer 3	90.28	1.73	-	-
	Isomer 4	91.09	-1.17	-	-
	Isomer 5	92.49	-1.44	α -2,6	-
	Isomer 6	93.73	-1.49	α -2,6	-
	Isomer 7	95.27	-5.03	-	-

Table S2. Clinical characteristics of the melanoma patients. Staging was based on the 8th edition of the American Joint Committee on Cancer (AJCC) tumor, node, metastasis (TNM) classification system. Breslow thickness refers to the depth of the melanoma from the surface of the skin to the deepest point of the tumor measured under microscope. pT3: Breslow thickness of the primary tumor is between 2.0 and 4.0 mm, which is subdivided into T3a or T3b based upon the absence or presence of ulceration. pT4: Breslow thickness of the primary tumor is more than 4.0 mm, which is subdivided into T4a or T4b based upon the absence or presence of ulceration. NMM refers to nodular malignant melanoma and SSM to superficial spreading melanoma.

Cases	Status	Breslow thickness (mm)	S100B serum level (μ g/L)
1	MM (non classified) (pT4b)	4.53	0.05
2	NMM (pT3b)	3.28	0.06
3	NMM (pT4a)	7.70	0.06
4	SSM with secunder nodular component (pT4b)	5.50	0.04
5	NMM (pT4b)	9.30	0.16
6	MM (non classified) (pT3b)	2.54	0.05
7	SSM (pT4a)	4.80	0.06
8	SSM (pT4a)	6.30	0.02
9	NMM (pT4b)	5.15	0.13
10	MM (non classified) (pT3b)	2.40	0.34
11	NMM (pT4b)	11.00	0.80
12	NMM (pT4b)	13.00	1.90
13	SSM (pT3b)	3.93	0.04
14	NMM (pT4b)	7.50	0.05
15	NMM (pT4a)	4.50	22.24
16	NMM (pT4a)	5.50	1.33
17	NMM (pT4b)	7.60	0.08
18	NMM (pT4b)	4.33	0.03

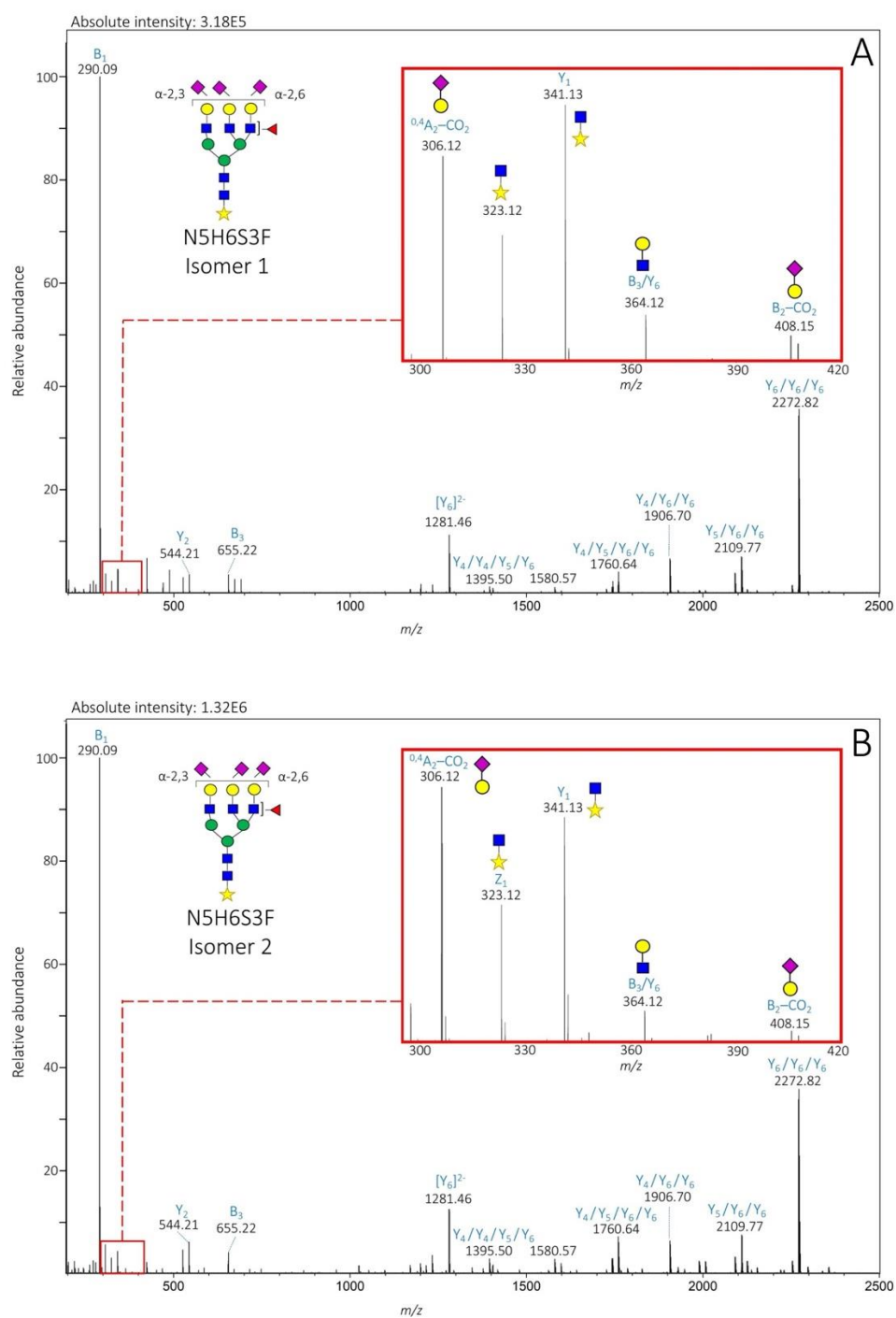


Figure S1. MS/MS spectra of isomers 1 (A) and 2 (B) of triantennary glycan N5H6S3F.

Both spectra contain diagnostic fragment ions m/z 306.12 and m/z 408.15 indicating the presence of α -2,6 and α -2,3 sialic acids. An increase in the ratio of α -2,6 to α -2,3 can be observed on the second spectra suggesting that two out of three sialic acids are α -2,3 linked in isomer 1 and two out of three are α -2,6 linked in isomer 2.

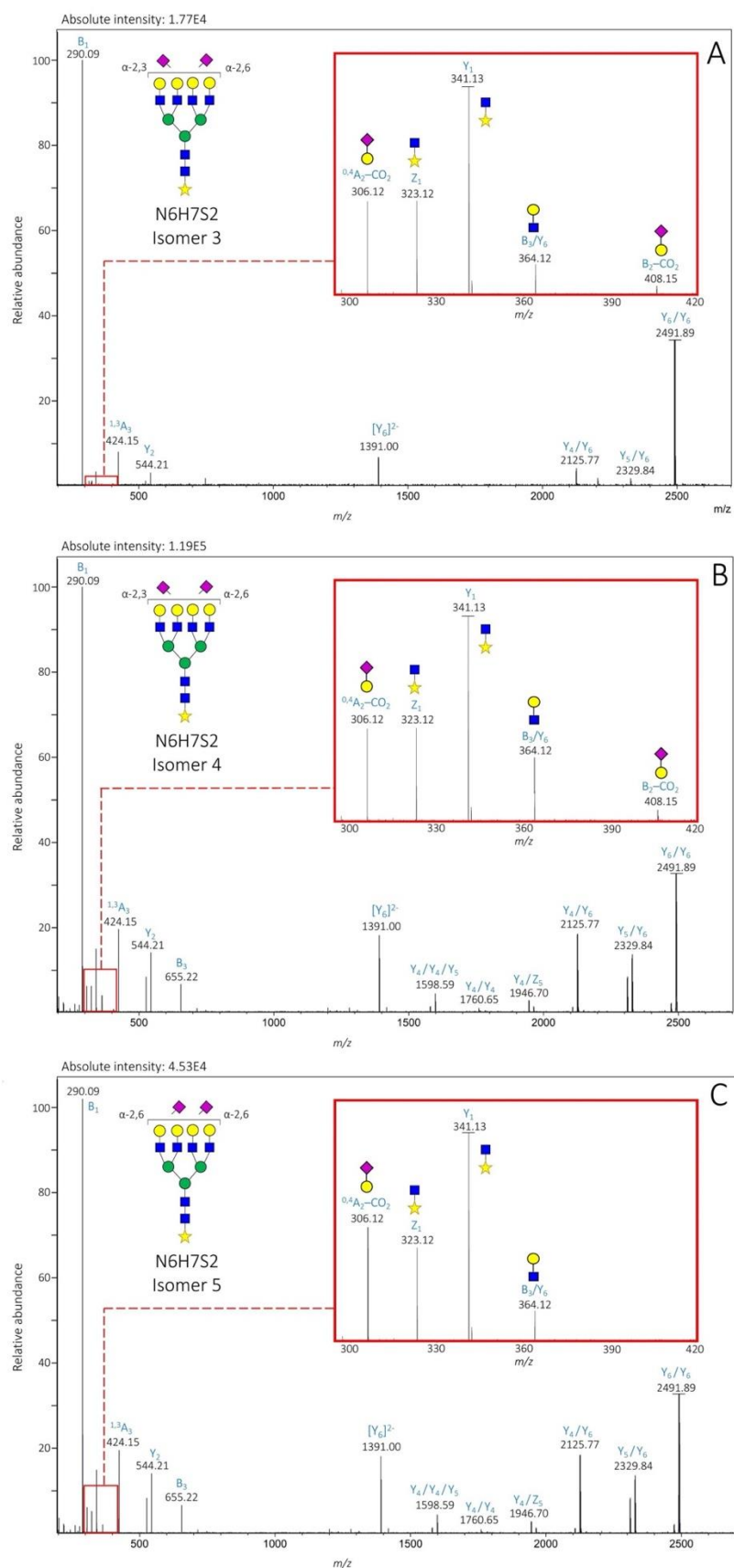


Figure S2. MS/MS spectra of isomers 3 (A), 4 (B) and 5 (C) of tetraantennary glycan N6H7S2.

Isomers 3 and 4 contain diagnostic fragment ions m/z 306.12 and m/z 408.15 indicating the presence of α -2,6 and α -2,3 linked sialic acids. The spectrum of isomer 5 lacks m/z 408.15 demonstrating that it is α -2,6 sialylated only.